Published in final edited form as:

J Periodontol. 2015 April ; 86(4): 595–605. doi:10.1902/jop.2015.140533.

Association of Serum 17 β -Estradiol Concentration, Hormone Therapy, and Alveolar Crest Height in Postmenopausal Women

Youjin Wang*, Michael J. LaMonte*, Kathleen M. Hovey*, Xiaodan Mai*, Mine Tezal†, Amy E. Millen*, Heather M. Ochs-Balcom*, Robert J. Genco†, Vanessa M. Barnabei‡, and Jean Wactawski-Wende*

* Department of Epidemiology and Environmental Health, School of Public Health and Health Professions, University at Buffalo, The State University of New York, Buffalo, NY

† Department of Oral Biology, School of Dental Medicine, University at Buffalo, The State University of New York

‡ Department of Obstetrics and Gynecology, School of Medicine and Biomedical Sciences, University at Buffalo, The State University of New York

Abstract

Background—Declines in endogenous estrogen levels after menopause can lead to systemic bone loss, including loss of oral bone and alveolar crest height (ACH). However, few studies have assessed both serum 17 β -estradiol (E₂) and exogenous hormone therapy (HT) use in relation to oral bone loss.

Methods—This study examines the associations among serum E₂, HT use, and ACH in 613 postmenopausal women from the Buffalo OsteoPerio study. Baseline ACH levels and 5-year ACH were assessed for groups according to E₂ level (undetectable, >5.00 to 18.00, >18.00 to 46.07, and >46.07 pg/mL) and among HT use (never, ever) using analysis of variance and analysis of covariance. Logistic regression was used to analyze the association of ACH loss with serum E₂ and HT use.

Results—In cross-sectional analyses, no association was found of serum E₂ with whole-mouth mean or worst-site ACH. However, history of HT use was associated with ACH. Women who had never used HT had more ACH loss assessed as a whole-mouth mean ACH ($P = 0.01$) and as worst-site ACH loss ($P = 0.03$). In logistic regression analyses of baseline ACH loss severity, HT never-users had two-fold higher odds of being in the severe ACH loss category compared to ever-users (odds ratio, 2.00; 95% confidence interval, 1.11 to 3.62). No association was observed of 5-year change in ACH with baseline serum E₂ or HT use.

Conclusion—Although this study did not detect an association with current serum E₂ level and ACH, HT use was found to be associated with less ACH loss in postmenopausal women.

Keywords

Epidemiology; osteoporosis; periodontitis; women's health

During the menopausal transition, levels of endogenous estrogen decline rapidly.^{1,2} For postmenopausal women, decreased estrogen concentrations have been shown to be associated with risk of systemic bone loss³⁻⁵ likely through increased production of osteoclasts favoring bone resorption over bone formation.⁵

Bone in the oral cavity has been far less extensively studied. The present authors have shown that postmenopausal women with osteoporosis have worse alveolar crest height (ACH) oral bone measures.⁶ A similar association has also been reported in a study of Jordanian post-menopausal women.⁷ It is reasonable to hypothesize that lower serum estrogen levels that lead to osteoporosis and systemic bone loss could also be associated with oral alveolar bone loss surrounding the teeth in postmenopausal women.

Studies have assessed the association of postmenopausal hormone therapy (HT) and alveolar crestal bone loss, although the findings are somewhat mixed.⁸⁻¹² Taguchi et al. reported no significant difference in oral bone height between estrogen users and non-users.⁹ In a 3-year randomized trial including 135 postmenopausal women, Civitelli et al. reported that HT significantly increased alveolar crestal bone mass ($P = 0.04$) and improved ACH but not significantly ($P = 0.34$).¹¹ Although the difference was statistically insignificant, a cross-sectional analysis of 213 women by Norderyd et al. found that estrogen users had slightly lower mean ($\pm$ SD) alveolar bone height than controls (2.7 ± 0.9 versus 2.8 ± 1.3 mm).¹² Payne et al. reported that individuals with deficient serum 17β -estradiol (E_2) concentrations had a higher frequency of greater ACH loss compared with E_2 -sufficient individuals in a 1-year longitudinal study with 24 postmenopausal women.¹⁰ The findings from previous studies have been hindered by small sample sizes and differences in the demographic makeup of the study populations. In addition, studies with quantitative measures of serum estradiol levels are lacking. To the best of the authors' knowledge, no other studies have investigated the associations of both serum estrogen levels and exogenous HT use with oral ACH levels in a single, large well-characterized cohort of post-menopausal women.

In the present study, the authors hypothesize that women with low serum E_2 levels may be predisposed to alveolar bone loss and that postmenopausal HT use may be associated with less oral bone loss. The study evaluates the cross-sectional relationships of measured serum E_2 concentrations, as well as HT use, with ACH level. The authors also examine the longitudinal associations of serum E_2 and HT use at baseline with ACH loss during a 5-year follow-up interval.

MATERIALS AND METHODS

Study Participants

Participants were recruited from the Women's Health Initiative Observational Study (WHI-OS) who were enrolled at the Buffalo Center Clinic, Buffalo, New York.¹³ A total of 1,341 postmenopausal women from the Buffalo WHI-OS participated in the Osteo-Perio study, an

ancillary study to the WHI-OS. The purpose of the OsteoPerio study was to investigate the association between osteoporosis and oral bone loss.^{6,13} The participants were enrolled and completed baseline visits from 1997 to 2001. Of 1,341 participants, 1,025 women returned about 5 years later for a follow-up visit (2002 to 2006). Participants were excluded from entering the baseline study if they had 1) <6 teeth present; 2) history of bone disease other than osteoporosis; 3) bilateral hip replacement; 4) cancer diagnosed in the past 10 years; or 5) any other serious illness.^{6,14} Participants were excluded from the follow-up study if they developed cancer or immunosuppressive disease during the follow-up or were exposed to long-term use of antibiotics.¹⁴ Among the participants who returned for follow-up, 620 had stored blood samples available for assessment of serum reproductive hormone levels. Of these, five reported having never used HT yet had E₂ levels >45 pg/mL, and two had incomplete ACH measurements at the follow-up examination. The present analyses are based on the remaining 613 participants (age range: 53.3 to 83.1 years; mean age: 65.5 ± 6.7 years). All the study participants provided written informed consent. Both the WHI-OS and the OsteoPerio Study were approved by the Health Sciences Institutional Review Board of the University at Buffalo.

ACH

ACH was measured for each tooth present in up to 48 sites from 24 teeth except for third molars and canines. Each tooth had both mesial and distal sites assessed. Up to 11 standardized intra-oral bitewing radiographs[§] including four vertical views were taken with exposure parameters specified (65 kVP, 100 mA) and time ranging from 0.1 to 0.45 seconds at a 150-cm focus to film distance.⁶ To control projection geometry of images, each participant's head was stabilized with a cephalometric head positioner. The angle between the mid-sagittal plane of the head and the tube head was constant at 18 degrees for all radiographs.

Radiographic and digitalized ACH assessment followed the method described by Hausmann and colleagues.¹⁵⁻¹⁷ ACH was defined as the distance in millimeters between the cemento-enamel junction (CEJ) and the most coronal part of the alveolar crest directly adjacent to the root surface along the long axis of the tooth. If a vertical defect was present, ACH was measured as the distance between the CEJ and the point directly adjacent to the root surface at the base of the defect for mesial and distal parts per tooth. The intra-observer coefficient of variation was 5.1%, and intraclass correlation was 0.98 for repeated ACH measures.^{14,18} Whole-mouth mean ACH was calculated by averaging the ACH levels in all teeth measured in the mouth. Larger ACH values indicate worse alveolar bone measures (more loss).

Five-year changes in ACH from baseline were calculated by side-by-side comparison of paired digitized images for each site.^{14,19} The baseline and follow-up images of the same individual at the same site were displayed side by side on the monitor and aligned together for comparison using a flicker system, and the examiners were masked to the exposure status. The difference from baseline was recorded as the change in ACH, with a negative

[§]Bennett HFQ 300 high-frequency x-ray generator, Bennett X-Ray Corp., Copiague, NY.

value indicating loss. All sites measured were averaged to construct a measure of whole-mouth mean change. The largest change over 5 years was used for worst-site change.

In addition to the continuous measures, the authors evaluated the relationships using ACH severity categories that were based on whole-mouth mean ACH measures and reported tooth loss due to periodontal disease.⁶ The three severity categories include 1) none (whole-mouth mean ACH <2 mm with no site ≥4 mm and no reported tooth loss due to periodontal disease); 2) mild/moderate (whole-mouth mean ACH ≥2 mm but <3 mm or 1 site ≥4 mm, but no tooth loss due to periodontal disease); and 3) severe (whole-mouth mean ACH ≥3 mm or 2 sites ≥5 mm, or any reported tooth loss due to periodontal disease).

Serum Estradiol

Fasting blood samples were collected from each participant by venipuncture before ACH measurement the morning of the baseline visits. Samples were processed within 30 minutes of collection and placed in liquid nitrogen (−196°) for long-term storage. Samples were later pulled and placed in freezers at −80°C until they were shipped for testing on dry ice. None of the samples were previously thawed. Serum E₂ concentration was measured by competitive electrochemiluminescence immunoassay^{||} at the Clinical & Epidemiologic Research Laboratory (Children's Hospital, Boston, Massachusetts). The lowest detection limit of this assay is 5 pg/mL, and the day-to-day imprecision values at concentrations of 34.8, 108, and 1,385 pg/mL are 5.2%, 2.9%, and 2.3%, respectively. A total of 131 participants had serum E₂ levels below the minimum detection level and were included in the analyses as a separate group. Participants with detectable serum E₂ levels were then divided into tertiles for analysis: 5.01 to 18.00 pg/mL (n = 160), 18.01 to 46.07 pg/mL (n = 161), and >46.07 pg/mL (n = 161).

Postmenopausal HT Use

Participants completed questionnaires asking about HT use history, including both estrogen and progestin at the WHI baseline visit. Details on type of HT used and years started and stopped were recorded for both current and past use. Participants also completed a current medication inventory in the clinic at baseline and at the 5-year follow-up visit. They were asked to bring all prescription and over-the-counter medications they were currently taking. Information about the name, frequency, and dose of the medications were recorded by research staff. For these analyses, postmenopausal HT use was considered as systemic use of estrogen alone or estrogen plus progestin. Participants were categorized into HT never- or ever-users and further categorized into never, former, or current users.

Other Health-Related Variables

Information on demographics, lifestyle, health, and dental hygiene practice were obtained from the baseline WHI-OS questionnaires and data collected during the OsteoPerio clinic visits. The following factors were assessed as potential confounders in the present analysis: 1) age (years); 2) body mass index (BMI) (kg/m²); 3) race/ethnicity (white/non-white); 4) smoking status (ever/never); 5) daily total calcium (mg) and vitamin D (IU) intake based on

^{||}Roche E Modular system, Roche Diagnostics, Indianapolis, IN.

dietary and supplement use; 6) hysterectomy (yes/no); 7) bilateral oophorectomy (yes/no); 8) history of diagnosed/treated diabetes (yes/no); 9) baseline HT use status (never, former, current; for serum E₂ analyses only); 10) years since menopause; 11) years post-menopause without using HT; 12) frequency of toothbrushing (<2/ 2 times/day); 13) flossing (weekly or less, more than weekly but less than daily, daily); 14) dental visits (never or only with problem/more than annually); and 15) number of teeth present. The reliability of the total calcium and vitamin D intakes is high (intraclass correlation ≈ 0.76 each).²⁰

Statistical Analyses

Participant characteristics were described according to categories of E₂ level (undetectable and tertiles of detectable E₂ concentrations) and HT use (never, ever) status at baseline. Differences were compared using analysis of variance (ANOVA), *t* test, and Pearson χ^2 test.

The assumptions for statistical analyses were checked with graphic diagnostics, and none of them were violated. In the analysis of ACH as a continuous measure, baseline whole-mouth mean, worst-site ACH values, and 5-year changes in ACH from baseline were compared among categories of E₂ level and HT use status at baseline using ANOVA and analysis of covariance (ANCOVA) with Bonferroni post hoc pairwise comparisons.

A multinomial logistic regression was used to assess the association among ACH severity and E₂ level category and HT use status at baseline. Odds ratio (OR) and 95% confidence interval (CI) were estimated for mild/moderate and severe ACH category. In the analysis of serum E₂, participants in the highest category served as the referent group. In the analyses of HT use, ever-users and current users were used as the referent groups.

Potential confounders that changed the point estimate in unadjusted models by 10% or that have biologically plausible associations were considered in the multivariate models. Here, final multivariate models were adjusted for age, BMI, smoking status, and years postmenopause without HT in the HT use status analyses. In the analyses of E₂ levels, baseline HT use status was also added in the final model.

All statistical analyses were conducted using software.[¶] All *P* values reported were two-sided, and statistical significance was defined as *P* < 0.05.

RESULTS

Baseline Characteristics

Baseline characteristics of study participants by E₂ categories and also by HT status are presented in Table 1. The participants were on average 65.5 years old and predominantly white (98.7%), and only 3.4% had known diabetes. Participants with serum E₂ levels >18 pg/mL were younger and more likely to be current HT users, more likely to have had a hysterectomy or bilateral oophorectomy, and had fewer years without postmenopausal HT compared with women with serum levels ≤ 18 pg/mL. With regard to HT use, ever-users were significantly younger than never-users (64.8 versus 67.1 years, respectively). Ever-

[¶]SAS v.9.3, SAS Institute, Cary, NC.

users were also more likely to have had hysterectomy and bilateral oophorectomy. Never-users had lower daily total calcium intake than ever-users, as assessed via a food frequency questionnaire and a medication inventory. No differences were observed in frequency of toothbrushing, flossing, or dental visits or number of teeth present according to serum E₂ levels or HT use status at baseline.

Cross-Sectional Analyses of Baseline ACH

The overall means of whole-mouth mean and worst-site ACH of the participants were 2.36 ± 0.73 and 4.50 ± 1.54 mm, respectively. Figure 1 presents baseline ACH measures for whole-mouth mean and worst-site ACH by serum E₂ levels and HT use status. No statistically significant differences were observed in whole-mouth mean ($P = 0.32$) or worst-site ($P = 0.23$) ACH levels across serum E₂ levels; however, in the comparison of HT never- and ever-users, baseline whole-mouth mean and worst-site ACH in never-users were significantly higher than in ever-users (adjusted mean $\pm$ SEM, whole-mouth mean 2.49 ± 0.05 versus 2.32 ± 0.03 mm, $P = 0.01$; worst site 4.73 ± 0.12 versus 4.42 ± 0.08 mm, $P = 0.03$).

The authors also compared never, former, and current HT users (data not shown) and observed statistically significant differences in whole-mouth mean (ANCOVA $P = 0.01$) and worst-site (ANCOVA $P = 0.03$) ACH among HT use categories. In pairwise comparison with Bonferroni correction, ACH values for never-users were greater than for former users (adjusted mean, whole-mouth mean 2.48 ± 0.05 versus 2.24 ± 0.07 mm, $P = 0.01$; worst site 4.71 ± 0.12 versus 4.22 ± 0.15 mm, $P = 0.02$), indicating worse oral alveolar bone status. A similar pattern was observed in current users compared to never-users; however, this difference did not reach statistical significance.

Table 2 presents the logistic regression analyses of the association of ACH severity with both serum E₂ levels and HT use at baseline. In unadjusted models, the authors observed increased odds of having mild/moderate (OR, 1.83; 95% CI, 1.03 to 3.25) and severe (OR, 2.13; 95% CI, 1.07 to 4.26) ACH loss among women with undetectable serum E₂ levels compared to the women in the highest E₂ level category (>46.07 pg/mL). However, after adjustment for age, BMI, smoking status, baseline HT status, and years without HT, these associations were no longer statistically significant.

HT never-users had increased odds of mild/moderate ACH in unadjusted (OR, 1.84; 95% CI, 1.19 to 2.86) and age-adjusted (OR, 1.64; 95% CI, 1.05 to 2.56) models compared with ever-users. Although additional adjustment reduced this association to non-significance, never-users still showed a sizable positive association with mild/moderate ACH in the multivariate adjusted model (OR, 1.51; 95% CI, 0.92 to 2.47). The odds of having severe ACH loss for never-users were significantly higher than for ever-users in all models examined (multivariate adjusted OR, 2.00; 95% CI, 1.11 to 3.62).

In the analyses of the association among the three HT use categories (never, former, current users) and ACH severity, never-users had significantly increased odds of having mild/moderate ACH than current users in unadjusted (OR, 1.90; 95% CI, 1.20 to 3.00) and age-adjusted (OR, 1.64; 95% CI, 1.03 to 2.62) models (data not shown). However, the

association became statistically insignificant after the additional multivariate adjustment (OR, 1.42; 95% CI, 0.83 to 2.44). The odds of having severe ACH for never-users were significantly higher than for current users in the unadjusted model (OR, 2.01; 95% CI, 1.19 to 3.41). The authors still observed positive associations after multivariate adjustment; however, the results were statistically insignificant. In contrast to never-users, former users showed no significant difference in the odds of having mild/moderate or severe ACH loss compared with current users.

Longitudinal Analyses of 5-Year Changes in ACH

Table 3 shows 5-year changes in whole-mouth mean and worst-site ACH by serum E₂ level and HT use status at baseline. The overall means of whole-mouth mean and worst-site ACH changes of the participants were -0.19 ± 0.22 and -1.14 ± 1.10 mm, respectively. The authors observed no differences in ACH progression from baseline to follow-up according to measured E₂ level or HT categories. Five-year average change in ACH was ≈ 0.2 mm for whole-mouth mean and 1 mm even at the worst site, irrespective of serum E₂ level or HT status.

DISCUSSION

In the present analysis of 613 postmenopausal women, the authors observed that history of HT use is associated with oral bone loss, whereas serum E₂ levels, measured at the same time of oral examination, have no association. To the best of the authors' knowledge, no other previous studies have examined the associations of ACH with both quantitative measures of serum E₂ concentrations and use of exogenous HT simultaneously. The results from this study suggest that HT use has a protective effect on alveolar bone loss in postmenopausal women. The finding may also suggest that a single measure of serum E₂ level is less predictive of oral bone loss than history of HT use.

A few longitudinal studies have examined the association between serum E₂ level and alveolar bone loss among postmenopausal women. Payne et al.¹⁰ conducted a 1-year longitudinal study with 24 post-menopausal women with a history of periodontitis, which consisted of 10 E₂-sufficient women who were undergoing estrogen therapy (serum E₂ >40 pg/mL) and 14 E₂-deficient women (<30 pg/mL). In the study, E₂ deficient women showed a higher frequency of significant ACH loss (bone height loss >0.3 mm) in total sites measured, compared with those with sufficient E₂ levels, although the difference was not statistically significant (4.8% versus 1.8%, $P > 0.05$).¹⁰ The study also reported that when alveolar bone change was assessed with computer-assisted densitometric image analysis (CADIA), E₂-sufficient women showed increased alveolar bone density over the 1-year period, whereas loss of alveolar bone density was observed among E₂-deficient women (CADIA mean $\pm$ SEM, 0.03 ± 0.07 versus -0.44 ± 0.07 , $P < 0.001$). However, considering the fact that the E₂-sufficient participants in the study were on estrogen therapy, the observed protective effect of higher serum E₂ concentrations on alveolar crestal density may have resulted from participants' HT use. The results from a different 2-year longitudinal study with 38 currently non-smoking postmenopausal women with a history of periodontitis by the same authors showed statistically insignificant differences in percentage of sites with

ACH loss (bone height loss >0.4 mm) between E₂-deficient (<30 pg/mL) and -sufficient (serum E₂ >40 pg/mL) women (8.1% versus 7.3%, $P > 0.05$).⁸ However, the crude categorization into E₂-deficient and -sufficient groups might have resulted in a loss of information on the variation of the risk within each category.²¹ The previous studies were also limited by small sample sizes and configuration of sample characteristics. Therefore, it is hard to generalize the findings from the studies.

The association of estrogen with alveolar bone loss and its possible mechanisms are not completely understood. Genco and Grossi have proposed a model on how estrogen deficiency could be a risk factor for periodontal disease.²² In the model, estrogen deficiency, along with periodontal bacterial bone-resorbing factors, leads to synthesis and secretion of proinflammatory cytokines such as interleukin (IL)-1 β and tumor necrosis factor (TNF)- α . Together with the bone-resorbing activity of TNF- α , IL-1 β promotes synthesis of IL-6, macrophage colony-stimulating factor, and granulocyte-macrophage colony-stimulating factor, which regulate osteoclast precursor proliferation and osteoclast activation. This cascade results in increased inflammation and alveolar bone resorption.

Optimal serum estrogen levels with HT for clinical effects on systemic bone health have been studied. Rapuri et al. reported that women with serum total estradiol concentrations >13 pg/mL showed higher femoral, total-body, and spine bone mineral density than those with concentrations <9 pg/mL.²³ Similarly, Ettinger et al. found that women whose serum estradiol levels were 10 to 25 pg/mL showed higher total hip, calcaneus, proximal radius, and spine bone mineral density than those with estradiol levels <5 pg/mL.²⁴ However, serum estrogen levels in response to HT vary by an individual's physiologic factors as well as daily variation of environmental factors, causing large inter- and intrapersonal variation in bioavailability of the drug.²⁵⁻²⁹ Therefore, measurement of a single serum E₂ level may not truly reflect an individual's profile over time. Moreover, whereas serum E₂ level in relation to systemic bone density is known, less is understood about how these levels are associated with oral bone loss, especially measures of ACH. It is also not clear whether current E₂ levels reflect past levels that may have an impact on the oral bone loss observed.

Although postmenopausal HT was associated with reduced odds of ACH severity in the present study, the authors did not observe the hypothesized association between serum E₂ concentration and ACH, possibly because of both inter- and intra-individual variation in estrogen bioavailability. This may be interpreted as HT use representing a longer and more generalized increase in bioavailable estrogen over time, and better reflecting impact on ACH and oral bone than a one-time measure of serum estradiol concentration. Further study with serial measures of serum E₂ may help us better understand this observed lack of association of serum E₂ level with ACH.

The authors did not observe a statistically significant association of 5-year change in ACH with baseline serum E₂ level or HT use. Findings in cross-sectional and longitudinal analyses were not completely aligned, for reasons that are not clear. Given the limited change detected in these women over the 5 years of follow-up, the ability to demonstrate change may have been limited. It is also possible that additional serial measures of estradiol and ACH are necessary to evaluate longitudinal patterns of change in these factors with

greater precision. Additional studies are needed to clarify this important issue in an aging population of postmenopausal women.

The accuracy and reliability of ACH measurement from two-dimensional radiographs are supported by extensive literature, and they have been widely used as an established diagnostic measure of periodontitis.^{16,30-35} In this method, usually interproximal sites are measured to increase accuracy and reliability, since the mid-buccal/lingual sites overlap with the tooth. It is well known that radiographs underestimate the true amount of bone loss.³⁰ Nevertheless, since the same method was used to measure ACH for comparison groups, all factors related to accuracy or reliability would lead to only non-differential misclassification, and therefore underestimation of the true effect.^{36,37}

The strengths of this study include the large sample size and concomitant assessment of serum E₂ concentration and exogenous HT use. To the authors' knowledge, this is one of the largest studies to date to assess the association between serum E₂ concentration and oral bone loss. Both cross-sectional and longitudinal analyses of ACH in relation to baseline serum E₂ level and HT use is another strength of this study. Finally, comprehensive assessment of demographics, lifestyle, and health factors as well as dental hygiene practices enabled us to assess the potential confounding effects of those in the analyses.

The present study also has several limitations. First, the study lacks serial measures of serum E₂ concentrations. A single baseline measurement of serum E₂ levels may not adequately represent the participants' long-term average hormone levels, which could limit the ability to detect associations with ACH in the cross-sectional assessment. To investigate the role of serum estrogen level on ACH loss, it is necessary to conduct prospective studies with serial measurements of serum estrogen concentrations. However, by collecting retrospective information on HT use, the study was able to provide evidence that postmenopausal HT use was associated with decreased ACH loss. The study participants are an average of 65.5 years old, and the majority of them are white. Therefore, the results may not be generalized to the entire postmenopausal population. An additional limitation that should be considered is potential misclassification of HT use. However, any misclassification is likely to be non-differential because study participants were unaware of their ACH status at the time of data collection. In addition, women were asked to bring in their current medications, and those with former use were helped by picture aids of HT medications. One might expect that HT users are more likely to be health conscious than never-users and therefore have better periodontal outcomes. However, women in this study did not show any significant differences in their oral hygiene practices according to HT use. Finally, although the authors assessed the potential confounders and adjusted them in the analysis, residual confounding may have occurred by other unmeasured factors.

CONCLUSIONS

HT use is associated with decreased likelihood of ACH loss among postmenopausal women. However, current serum E₂ concentration is not associated with ACH. Longitudinal studies of changes in ACH with serial assessment of serum E₂ level may help better understand these findings and define the role of serum estrogen levels in oral bone loss over time.

ACKNOWLEDGMENTS

Program Office: (National Heart, Lung, and Blood Institute, Bethesda, Maryland) Jacques Rossouw, Shari Ludlam, Dale Burwen, Joan McGowan, Leslie Ford, and Nancy Geller. Clinical Coordinating Center: (Fred Hutchinson Cancer Research Center, Seattle, Washington) Garnet Anderson, Ross Prentice, Andrea LaCroix, and Charles Kooperberg. Investigators and Academic Centers: (Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts) JoAnn E. Manson, (MedStar Health Research Institute/Howard University, Washington, DC) Barbara V. Howard, (Stanford Prevention Research Center, Stanford, California) Marcia L. Stefanick, (The Ohio State University, Columbus, Ohio) Rebecca Jackson, (University of Arizona, Tucson/Phoenix, Arizona) Cynthia A. Thomson, (University at Buffalo, Buffalo, New York) Jean Wactawski-Wende, (University of Florida, Gainesville/Jacksonville, Florida) Marian Limacher, (University of Iowa, Iowa City/Davenport, Iowa) Robert Wallace, (University of Pittsburgh, Pittsburgh, Pennsylvania) Lewis Kuller, (Wake Forest University School of Medicine, Winston-Salem, North Carolina) Sally Shumaker. Women's Health Initiative Memory Study: (Wake Forest University School of Medicine, Winston-Salem, North Carolina) Sally Shumaker. This study was supported by grant IR01DE13505 from the National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH), Bethesda, Maryland, (awarded to JW-W), grant OS950077 from U.S. Army, Medical Research and Materiel Command, Fort Detrick (awarded to JW-W), and the National Heart, Lung, and Blood Institute (NHLBI), NIH, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. The authors report no conflicts of interest related to this study.

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Figure 1.

Cross-sectional association of serum E_2 level, HT use status at baseline, and ACH (N = 613). **A)** Adjusted mean ACH at baseline by serum estradiol level, adjusted for age, BMI, smoking status, years postmenopause without HT, and baseline HT use status. **B)** Adjusted mean ACH at baseline by HT use, adjusted for age, BMI, smoking status, and years postmenopause without HT. ND = non-detectable. T1: >5.00, 18.00 pg/mL; T2: >18.00, 46.07 pg/mL; T3: >46.07 pg/mL.

Table 1

Characteristics of Postmenopausal Women [mean (SD) or n (%)] by Categories of Serum E2 Level and HT Use Status at Baseline: The Buffalo OsteoPerio Study, 1997 to 2001 (N = 613)

Characteristic	All	Serum E ₂ Level Categories at Baseline					HT Use Status at Baseline		
		Undetectable	>5.00 and pg/mL	18.00 pg/mL	>18.00 and pg/mL	>46.07 pg/mL	Never-Users	Ever-Users	* p
n	613	131	160	161	161	161	188	425	
Age at baseline (years)	65.5 (6.7)	66.6 (6.7)	66.2 (6.9)	64.8 (6.8)	64.6 (6.1)	64.8 (6.5)	67.1 (6.9)	64.8 (6.5)	<0.01
Race (white)	605 (98.7)	129 (98.5)	158 (98.8)	159 (98.8)	159 (98.8)	159 (98.8)	186 (98.9)	419 (98.6)	>0.99 [†]
Cigarette smoking									
Never	332 (54.2)	75 (57.3)	80 (50.0)	82 (50.9)	95 (59.0)	224 (52.7)	108 (57.4)	224 (52.7)	0.28
Ever	281 (45.8)	56 (42.7)	80 (50.0)	79 (49.1)	66 (41.0)	201 (47.3)	80 (42.6)	201 (47.3)	
Daily total calcium Intake (mg) [‡]	1,423.2 (740.3)	1,357.1 (860.2)	1,453.9 (708.1)	1,468.8 (697.5)	1,400.9 (709.1)	1,484.5 (753.3)	1,284.8 (692.1)	1,484.5 (753.3)	<0.01
Daily total vitamin D Intake (IU) [‡]	620.2 (2,044.6)	530.3 (350.2)	541.0 (306.0)	553.0 (292.8)	838.6 (3,952.3)	673.5 (2,446.7)	499.8 (311.1)	673.5 (2,446.7)	0.15
Hormone therapy									
Never	188 (30.7)	78 (59.5)	89 (55.6)	21 (13.0)	0				<0.01
Former	107 (17.5)	43 (32.8)	45 (28.1)	15 (9.3)	4 (2.5)				
Current	318 (51.9)	10 (7.6)	26 (16.3)	125 (77.6)	157 (97.5)				
Hysterectomy	220 (35.9)	36 (27.5)	39 (24.4)	67 (41.6)	78 (48.5)	182 (42.8)	38 (20.2)	182 (42.8)	<0.01
Bilateral oophorectomy [§]	93 (15.2)	11 (8.4)	21 (13.1)	31 (19.5)	30 (18.6)	77 (18.2)	16 (8.5)	77 (18.2)	<0.01
Years since menopause	17.4 (8.4)	18.1 (8.9)	17.5 (8.5)	17.2 (8.3)	17.0 (8.3)	17.2 (8.5)	17.9 (8.3)	17.2 (8.5)	0.36
Years without HT	11.7 (9.6)	16.0 (9.3)	15.1 (9.0)	9.0 (8.7)	7.7 (8.6)	9.1 (8.8)	17.8 (8.4)	9.1 (8.8)	<0.01
BNI (kg/m ²)	26.5 (4.9)	26.2 (4.6)	26.7 (4.8)	27.0 (5.3)	26.0 (4.9)	26.2 (4.8)	27.1 (5.1)	26.2 (4.8)	0.05
Diabetes history	21 (3.4)	2 (1.5)	6 (3.8)	11 (6.8)	2 (1.2)	17 (4.0)	4 (2.1)	17 (4.0)	0.24
Toothbrushing									
<2 times/day	144 (23.5)	37 (28.2)	37 (23.1)	31 (19.3)	39 (24.2)	101 (23.8)	43 (22.9)	101 (23.8)	0.81
2 times/day	469 (76.5)	94 (71.8)	123 (76.9)	130 (80.7)	122 (75.8)	324 (76.2)	145 (77.1)	324 (76.2)	
Teeth flossing [‡]									
Once/week or less	173 (28.3)	43 (32.8)	42 (26.4)	45 (28.0)	43 (26.7)	113 (26.6)	60 (32.1)	113 (26.6)	0.34

Characteristic	Serum E ₂ Level Categories at Baseline					HT Use Status at Baseline		
	All	Undetectable	>5.00 and pg/mL	18.00 pg/mL	>18.00 and pg/mL	46.07 pg/mL	>46.07 pg/mL	* <i>p</i>
More than once/week and less than daily	181 (29.6)	35 (26.7)	51 (32.1)	50 (31.1)	45 (28.0)	131 (30.8)		
Every day	258 (42.2)	53 (40.5)	66 (41.5)	66 (41.0)	73 (45.3)	181 (42.6)		
Visit dentist								0.13
Never or only with problem	47 (7.7)	11 (8.4)	14 (8.8)	11 (6.8)	11 (6.8)	28 (6.6)		
Once a year or more	566 (92.3)	120 (91.6)	146 (91.3)	150 (93.2)	150 (93.2)	397 (93.4)		
Number of teeth present	23.8 (4.9)	24.1 (4.6)	23.7 (5.2)	23.6 (4.9)	23.9 (4.7)	24.1 (4.7)		0.07

Bold text indicates statistical significance.

* χ^2 test for categorical variables, ANOVA/*t* test for continuous variables.

[†] Fisher exact test.

[‡] One missing (n = 612).

[§] Three missing (n = 610).

Table 2

Logistic Regression Analyses of Baseline ACH Severity Category^{*} by Serum E₂ Level and HT Use Status at Baseline (N = 613)

Model	n	None (n = 170) (n)	Mild/Moderate (n = 308)			Severe (n = 135)		
			n	OR (95% CI)	P	n	OR (95% CI)	P
Serum E ₂ levelcategory at baseline								
Unadjusted								
>46.07 pg/mL	161	50	81	1.0		30	1.0	
> 18.00 and 46.07 pg/mL	161	49	72	0.91 (0.55 to 1.50)	0.71	40	1.36 (0.74 to 2.52)	0.33
>5.00 and 18.00 pg/mL	160	46	81	1.09 (0.66 to 1.80)	0.75	33	1.20 (0.63 to 2.26)	0.58
Undetectable	131	25	74	1.83 (1.03 to 3.25)	0.04	32	2.13 (1.07 to 4.26)	0.03
P for trend				0.04			0.07	
Age adjusted								
>46.07 pg/mL	161	50	81	1.0		30	1.0	
>18.00 and 46.07 pg/mL	161	49	72	0.91 (0.55 to 1.52)	0.72	40	1.36 (0.72 to 2.54)	0.34
>5.00 and 18.00 pg/mL	160	46	81	0.99 (0.59 to 1.66)	0.97	33	1.04 (0.54 to 1.99)	0.92
Undetectable	131	25	74	1.65 (0.92 to 2.96)	0.09	32	1.83 (0.90 to 3.71)	0.09
P for trend				0.13			0.23	
Multivariate adjusted [†]								
>46.07 pg/mL	161	50	81	1.0		30	1.0	
>18.00 and 46.07 pg/mL	161	49	72	0.86 (0.51 to 1.46)	0.57	40	1.23 (0.64 to 2.37)	0.54
>5.00 and 18.00 pg/mL	160	46	81	0.76 (0.38 to 1.53)	0.44	33	0.93 (0.38 to 2.28)	0.87
Undetectable	131	25	74	1.29 (0.59 to 2.86)	0.53	32	1.93 (0.72 to 5.16)	0.19
P for trend				0.64			0.24	
HT use status at baseline								
Unadjusted								
Ever-user	425	134	206	1.0		85	1.0	
Never-user	188	36	102	1.84 (1.19 to 2.86)	<0.01	50	2.19 (1.32 to 3.64)	<0.01
Age adjusted								
Ever-user	425	134	206	1.0		85	1.0	
Never-user	188	36	102	1.64 (1.05 to 2.56)	0.03	50	1.84 (1.09 to 3.10)	0.02
Multivariate adjusted [‡]								
Ever-user	425	134	206	1.0		85	1.0	
Never-user	188	36	102	1.51 (0.92 to 2.47)	0.10	50	2.00 (1.11 to 3.62)	0.02

Bold text indicates statistical significance.

^{*} Categorical measure of ACH severity:⁶ none (whole-mouth mean ACH <2 mm with no site 4 mm and no reported tooth loss due to periodontal disease); mild/moderate (whole-mouth mean ACH 2 mm but <3 mm, or 1 site 4 mm but no tooth loss due to periodontal disease); or severe (whole-mouth mean ACH 3 mm, or 2 sites 5 mm, or any reported tooth loss due to periodontal disease).

[†] Adjusted for age, BMI, smoking status (never, ever), baseline HT status (never, former, current), and years without HT.

[‡] Adjusted for age, BMI, smoking status (never, ever), and years without HT.

Table 3

Longitudinal Association (adjusted mean $\pm$ SEM) of Baseline E₂ Level, HT Use Status, and 5-Year Change in ACH (N = 613)

5-year change in ACH measures (mm)	Serum E ₂ Level Category at Baseline				HT Use Status at Baseline		
	Undetectable	>5.00 and 18.00 pg/mL	>18.00 and 46.07 pg/mL	>46.07 pg/mL	*† p	Never-Users	Ever-Users
n	131	160	161	161		188	425
Whole-mouth mean ACH change							
Age adjusted	-0.17 $\pm$ 0.02	-0.19 $\pm$ 0.02	-0.21 $\pm$ 0.02	-0.18 $\pm$ 0.02	0.45	-0.20 $\pm$ 0.02	-0.18 $\pm$ 0.01
Multivariate adjusted	-0.15 $\pm$ 0.02	-0.17 $\pm$ 0.02	-0.22 $\pm$ 0.02	-0.20 $\pm$ 0.03	0.18	-0.20 $\pm$ 0.02	-0.18 $\pm$ 0.01
Worst-site ACH change							
Age adjusted	-1.11 $\pm$ 0.10	-1.15 $\pm$ 0.09	-1.16 $\pm$ 0.09	-1.15 $\pm$ 0.09	0.98	-1.20 $\pm$ 0.08	-1.12 $\pm$ 0.05
Multivariate adjusted	-1.11 $\pm$ 0.10	-1.13 $\pm$ 0.09	-1.12 $\pm$ 0.11	-1.12 $\pm$ 0.12	>0.99	-1.21 $\pm$ 0.09	-1.12 $\pm$ 0.05

* ANCOVA adjusting for age.

† ANCOVA adjusting for age, BMI, smoking status (never, ever), years without HT, and baseline HT status (never, former, current).

‡ ANCOVA adjusting for age, BMI, smoking status (never, ever), and years without HT.